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(54) Title: MODULATION OF IMMUNE RESPONSE BY RIBAVIRIN

(57) Abstract

The response of an immune system to a challenge is modified by presenting the system with a nucleoside in a concentration selected to have an effect on a B7 marker that is inverse from the effect of the challenge. Contemplated challenges include allergens, neoplasm, virus, bacteria, infestation, and autoimmune reaction. Molecular markers of particular interest are B7–1 and B7–2. Preferred nucleosides are Ribavirin and Ribavirin analogs, especially provided within a concentration range between about 0.2 :M and about 5 :M, respectively, in a fluid containing cells expressing the B7 marker.

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MODULATION OF IMMUNE RESPONSE BY RIBAVIRIN

Field Of The Invention

The field of the invention is immunology.

Background Of The Invention

In addition to the commonly employed physiological and phenotypical diagnostic parameters, diseases can sometimes be correlated with molecular markers such as polidy, mutations in specific genes, display of distinct cell surface markers and so forth. Many of these markers act as disease-specific predictors or indicators, and can thus be used as a diagnostic tool for a clearly defined physiological condition.

In recent years, many attempts have been made to correlate relatively complex diseases such as autoimmunity, asthma, cancer etc. with specific molecular markers. For several studies have found a direct or indirect involvement of the costimulatory molecules B7-1 and B7-2 in modulating the immune system in diseases. However, despite many detailed insights into the various expression levels B7-1 and B7-2 in diseases obtained through such studies, a comprehensive and unified picture has not be elaborated. (Hepatology 25, No.5, 1997 p1108-1114: Expression of costimulatory molecules B7-1 and B7-2 and human hepatocellular carcinoma; J. Cancer Res. Clin. Oncol. 124, No.7, 1998 p383-388: Expression of costimulatory molecules B7-1 and B7-2 on human gastric carcinoma; J. Neuroimmunol. 84, No.2, 1998 p179-187: Costimulatory CD80 (B7-1) and CD86 (B7-2) on cerebrospinal fluid cells in multiple sclerosis; J Neuroimmunol 91, No1-2, 1998, p198-203: B7-1 (CD80), B7-2 (CD86), interleukin-12 and transforming growth factor-beta mRNA expression in CSF and peripheral blood mononuclear cells from multiple sclerosis patients).

In many instances, apparently inconsistent correlations have been observed between B7-1, B7-2 and specific diseases. See Figure 1. In some types of cancer, for example, B7-1 is present in relatively high amounts and B7-2 is present in relatively low amounts. In other types of cancers, B7-1 and B7-2 have exactly the opposite correlation. (J. Cancer Res. Clin. Oncol. 124, No.7 1998 p383-388: Expression of costimulatory molecules B7-1 and B7-2 on human gastric carcinoma; Br. J. Haematol 102, No.5, 1998 p1257-1262: The expression of costimulatory molecules and their relationship to the prognosis of human acute myeloid

leukemia: poor prognosis of B7-2-positive leukemia; Int. J. Mol. Med. 2, No.2, 1998 p167-171: Lack of B7-1 and B7-2 on head and neck cancer cells and possible significance for gene therapy).

B7-1 and B7-2 expression also show only inconsistent correlation with known cytokine patterns. See Figure 2. For example, enhanced expression of B7-1 has been correlated with both up- and down- regulation of Type 1 response, and B7-2 has also been correlated with both up- and down- regulation of Type 1 response. The same can be said for correlation with B7-1 and B7-2 with Type 2 response. (see Figure 1) (Am. J. Respir. Cell. Mol. Biol. 17, No.2, 1997 p235-242: Differential regulation of human, antigen-specific Type 1 and Type 2 responses by the B-7 homologues CD80 and CD86; J. Immunol. 156, No.8, 1996 p2387-2391: Costimulation of IL-4 production by murine B7-1 and B7-2 molecules.).

Still further, it is not clear which drugs or even drug categories would be effective in modulating B7-1 or B7-2 activity, and even if such drugs were identified, and it remains unclear how to beneficially make use of these costimulatory molecules to modulate the immune system. Taking together all of these unknowns, there is still a considerable need to provide methods and compositions for modulating one or more of the B7 markers, especially as a means of affecting the response of an immune system to a given challenge.

Brief Description Of The Drawings

Figure 1 is a table correlating specific diseases and their correlation with B7-1 and B7-2 expression.

Figure 2 is a table correlating various types of diseases with Type 1, Type 2, B7-1, and B7-2 expression.

Summary Of The Invention

This invention provides methods and compositions by which the response of an immune system to a challenge is modified. In general, the response is modified by presenting the system with a nucleoside in a concentration selected to have an effect on a B7 marker that is inverse from the effect of the challenge.

In one aspect of preferred embodiments, the challenges are selected from the groups consisting of allergens, neoplasm, virus, bacteria, infestation, and autoimmune reaction. Molecular markers of particular interest are B7-1 and B7-2. In another aspect of preferred embodiments the nucleoside is a Ribavirin analogs, and in especially preferred embodiments the nucleoside is Ribavirin. In yet another aspect of preferred embodiments sufficient nucleoside is provided to achieve a concentration range between about 0.2 :M and about 5 :M, respectively, in a fluid containing cells expressing the B7 marker.

In still another aspect of preferred embodiments, the challenge is correlated with an increase in Type 2 response, and application of the nucleoside is correlated with a decrease in Type 2 response.

Detailed Description Of Specific Embodiments

The present inventor has discovered that there is a surprising link between certain nucleosides, especially Ribavirin and its analogues, and expression of one or more of the B7 markers. Further discoveries revealed another unexpected link -- that application of such nucleosides can be used to favorably affect the outcome of a disease or other challenge. In particular, a method of modulating a response of an immune system to a challenge has been discovered comprising: (a) correlating the challenge with an effect on a B7 marker; (b) correlating application of a nucleoside within a concentration range with modulation of the B7 molecular marker that is inverse to the effect; and (c) presenting the immune system with the nucleoside within the concentration range.

As used herein, the term "nucleoside" refers to a compound composed of any pentose or modified pentose moiety attached to a specific position of a heterocycle or to the natural position of a purine (9-position) or pyrimidine (1-position) or to the equivalent position in an analog, including especially both D- and L- forms of nitrogenous bicyclic and monocyclic heterocycles. The term "D-nucleosides" refers to nucleoside compounds that have a D-ribose sugar moiety (e.g., Adenosine). The term "L-nucleosides" refers to nucleoside compounds that have an L-ribose sugar moiety. The term "nucleotide" means a nucleosides in which phosphate esters substituted on the 5' position of a nucleoside.

The term "pharmaceutically acceptable salts" refers to any salt derived from inorganic and organic acids or bases.

The term "neoplasm" refers broadly to any sort of autonomous morbid growth of tissue that may or may not become malignant, including all manner of tumors and cancers.

The terms "treating" or "treatment" of a disease refer to executing a protocol, which may include administering one or more drugs to a patient, in an effort to alleviate signs or symptoms of the disease. Thus, "treating" or "treatment" do not require complete alleviation of signs or symptoms, do not require a cure, and specifically include protocols which have only marginal effect (such as placebo effect) on the patient.

As used herein, the term "immune system" means any collection of immuno-competent cells that collectively identify and attack foreign entities, and that dynamically responds to new pathogens or other challenges. Examples of immune systems are human or other mammalian immune systems that include a spleen, thymus B-lymphocytes, T-lymphocytes and antibodies. An immune system as defined herein must have a cellular component, but may or may not have a humoral component. Where a humoral component is included in the immune system, the humoral component may include soluble molecules secreted from immunocompetent cells, including antibodies or interleukins. Examples for soluble molecules are IgG, IgM, IgE or IL2, IL4, IL10.

Under this definition, whole blood, as well as blood depleted of fibrinogen, platelets and erythrocytes is considered to comprise an immune system, since it contains immuno-competent cells that are capable of dynamically responding to new pathogens. Other immune systems are cell culture mediums containing immunocompetent cells. In contrast, a buffered solution of antibodies is not considered an immune system, since it does not contain a plurality of immunocompetent cells. In still other embodiments, human or other animals all contain immune systems as defined herein.

The term "challenge" is used herein to mean any component or event that provokes a response by the immune system. Challenges may be grouped in three categories: self, non-self and altered self challenges. Self-type challenges include cells or molecules, wherein the immune system and the challenge are from the same organism, self proteins or autologous proteins and fragments thereof. Examples include human blood cells, undifferentiated cells, antibodies or coagulation factors from the same human. Non-self-type challenges include cells, viruses or molecules, wherein the immune system and the

challenge are from different organisms, or the challenge is xenogenic. Examples include organs or cells from a non-identical donor, bacteria, viruses, or any type of molecules typical for other species, including endotoxins, enzymes or structural proteins. Altered self-type challenges include cells or molecules wherein the immune system and the challenge are from the same organism, but wherein the challenge is subject to modifications, degradative or neoplastic changes. Examples of such modifications include modifying the profile of a B7 marker on antigen presenting cells. Examples of degradative changes include cells committed to apoptosis or necrotic tissue. Examples of neoplastic changes induction of cancer.

The terms "immune system response" and "immune response" are used herein to mean any response of an immune system to a challenge. Of particular interest in this application are immune responses that include modulation of a B7 marker. Such modulation may comprise any combination of increase or decrease in B7-1 and B7-2 expression. Thus, all of the responses tabulated in the tables of Figures 1 and 2 are examples of contemplated immune system responses.

Other contemplated immune system responses include engagement of cellular components in cell specific interactions or changes in genetic activity. Cell specific interactions may be cell-cell interactions or cell-challenge interactions. Examples for cell-cell interactions are T-cells contacting T-helper cells or T-helper cells contacting macrophages. Examples for cell-challenge interactions are antigen presenting cells incorporating the challenge, processing the challenge and displaying the processed challenge on the cell surface, or B-cells displaying challenge specific antibodies on their cell surface and binding the challenge with the antibody. Changes in genetic activity may be rearrangements in genomic DNA, or selective activation of genes. Examples for rearrangements in genomic DNA are splicing events leading to affinity maturation of antibodies against the challenge or splicing events leading to a class switch between different classes of antibodies. Examples for selective activation of genes are increase or decrease of transcription or translation of genes coding for interleukins or B7-1 or B7-2.

As used herein, producing a B7 effect that is 'inverse" to the pattern associated with the challenge means that the B7 effect produced by the nucleoside alone is at least marginally in an opposite direction to that associated with the challenge alone. Thus, if the

challenge is associated with reduced B7-1 expression, an inverse B7 effect would be one in which B7-1 is at least marginally elevated. Similarly, if the challenge is associated with an increased B7-2 expression, an inverse B7 effect would be one in which B7-2 is at least marginally reduced.

As used herein the term "presenting the immune system with a nucleoside" means that the nucleoside sufficiently contacts some component of the immune system to produce an immune system response. In preferred embodiments this means adding the nucleoside to a body. In other embodiments this means adding the nucleoside to a vessel, or other container of the immune system.

It should be appreciated that the definition of term "presenting the immune system with a nucleoside" is sufficiently broad to include any combination of *in-vivo*, *in-vitro*, or *ex-vivo* contact. I*n-vivo* may include injection, ingestion, transdermal delivery or inhalation. Examples for various injection are intramuscular, intravenous, or subcutaneous injection. Examples for various forms of ingestion are tablets, syrups, or powders. Occlusive dressings, ointments or electrophoretic methods may achieve transdermal delivery. Inhalation may be encompass methods of vaporizing or spraying.

In-vitro contacting may be achieved by either dispensing a nucleoside containing solution to the immune system in a suitable vessel, or by dissolving the nucleoside in a solution that may or may not be part of the immune system. Examples for dispensing include automated or manual pipetting, dripping, pouring or injecting a nucleoside containing solution to the immune system. Alternatively, a nucleoside may also be dissolved in a fluid by stirring, mixing or pouring Ribavirin in the fluid. This fluid may comprise the immune system or may be a carrier solution including buffer, isotonic solutions, blood. This carrier may then be dispensed to the immune system.

Ex-vivo contacting may be achieved in several steps comprising (1) collecting part of the immune system from a source, (2) administering the nucleoside to the immune system and (3) returning the immune system at least in part to the source. Collecting part of the immune system may be done by retrieving part of the immune system from an *in-vivo* or *in-vitro* source. Examples of *in-vivo* sources are vertebrate animals, including humans, and invertebrate animals. Retrieving may be done by venipuncture, eye bleeds, or pinpricks.

Examples of *in-vitro* sources are cell cultures containing the immune system, treated or stored blood. The retrieving may be done by any means of fluid transfer, for example automated or manual pipetting, aspiration, dripping and so on. Returning the immune system to the source may be done by any means of fluid transfer. This may be in the case of an *in-vitro* source automated or manual pipetting, aspiration, dripping or in the case of an *in-vivo* source injecting intravenously.

Contemplated nucleosides are Ribavirin (1-β-D-Ribofuranosyl-1,2,4-Triazole-3-Carboxamide), and analogs thereof. To clarify matters, Ribavirin analogs means any derivatized Ribavirin in which (1) one or more of the hydroxyl groups is substituted by a non-hydroxyl moiety having less than 25 atoms, including H, lower alkyl, lower aryl, lower aralkyl, lower alkyl alkenyl, halogen, and so forth, and independently one or more of the hydrogens is substituted by a non-hydrogen moiety having less than 25 atoms, including OH, lower alkyl, lower aryl, lower aralkyl, lower alkyl alkenyl, halogen, and so forth.

The ribavirin, ribavirin analog, or other nucleoside is preferably formulated in a buffered aqueous solution. In alternative embodiments, however, the nucleoside may be formulated in many other liquid or solid forms. Liquid forms may be solutions comprising pure solvents including water, DMSO or ethanol. Liquid forms may also comprise solutions having mixtures of solvent with other solvents or dissolved solids including water-ethanol mixtures, water-DMSO mixtures, buffers. Furthermore, liquid forms of nucleosides may be mixed for example with consistency-modifying substances to form gels, creams or ointments. Examples are amphiphilic molecules, waxes or gelatin. Solid forms may comprise solids that may or may not be active ingredients. Examples for active ingredients are buffers, ion-exchange resins including MOPS, phosphates or citrates. Examples of inactive ingredients include starch, cellulose or silica. Furthermore, solid forms may be in various preparations, including tablets, capsules, powder etc.

In preferred embodiments, sufficient nucleoside is provided to achieve a concentration range between about .2 :M and about 5 :M, respectively, in a fluid containing cells expressing the B7 marker. Less preferred embodiments contemplate other concentrations within the range of 0.1 μM to about 10 μM.

In another aspect of preferred embodiments, the challenge is correlated with an increase in Type 2 response, and application of the nucleoside is correlated with a decrease in Type 2 response. Type 2 response can be understood as follows.

Mammalian immune systems contain two major classes of lymphocytes: B lymphocytes (B cells), which originate in the bone marrow; and T lymphocytes (T cells) which originate in the thymus. B cells are largely responsible for humoral immunity (i.e., antibody production), while T cells are largely responsible for cell-mediated immunity. T cells are generally considered to fall into two subclasses, helper T cells and cytotoxic T cells. Helper T cells activate other lymphocytes, including B cells and cytotoxic T cells, and macrophages, by releasing soluble protein mediators called cytokines that are involved in cell-mediated immunity. As used herein, lymphokines are a subset of cytokines.

Helper T cells are also generally considered to fall into two subclasses, Type 1 and Type 2. Type 1 cells (also known as Th1 cells) produce interleukin 2 (IL-2), tumor necrosis factor (TNFV) and interferon gamma (IFN(), and are responsible primarily for cell-mediated immunity such as delayed type hypersensitivity and antiviral immunity. In contrast, Type 2 cells (also known as Th2 cells) produce interleukins, IL4, IL-5, IL-6, IL-9, IL-10 and IL-13, and are primarily involved in assisting humoral immune responses such as those seen in response to allergens, e.g. IgE and IgG4 antibody isotype switching (Mosmann, 1989, *Annu Rev Immunol.* 7:145-173).

As used herein, the terms Type 1 and Type 2 "responses" are meant to include the entire range of effects resulting from induction of Type 1 and Type 2 lymphocytes, respectively. Among other things, such responses include variation in production of the corresponding cytokines through transcription, translation, secretion and possibly other mechanisms, increased proliferation of the corresponding lymphocytes, and other effects associated with increased production of cytokines, including motility effects.

As described in US Patent no. 5767097 to Tam (June 1998), the disclosure of which is incorporated herein by reference, either of Type 1 and Type 2 responses can be selectively suppressed while the other is either induced or left relatively unaffected, and either of Type 1 or Type 2 responses can be selectively induced while the other is either suppressed or left relatively unaffected. Also, as set forth in co-pending PCT application

no. PCT/US98/00634, the disclosure of which is incorporated herein by reference, certain nucleosid s such as ribavirin are effective in selectively modulating Type 1 and Type 2 responses relative to one another. Determination of which nucleosides are effective in reducing Type 2 response is readily determined by experimentation.

It is contemplated that the methods described herein may be used to treat a wide variety of diseases, , and in fact any disease which responds favorably to such treatment. Among other things it is specifically contemplated that such combinations may be used to treat an allergen (allergy), a neoplasm (cancer), a virus (viral infection), a bacterium (bacterial infection), an infestation, or an autoimmune disease.

Infections contemplated to be treated with the nucleosides of the present invention include respiratory syncytial virus (RSV), hepatitis B virus (HBV), hepatitis C virus (HCV), herpes simplex type 1 and 2, herpes genitalis, herpes keratitis, herpes encephalitis, herpes zoster, human immunodeficiency virus (HIV), influenza A virus, hantann virus (hemorrhagic fever), human papilloma virus (HPV), measles and fungus. It is especially contemplated that combinations claimed herein will be useful in treating chronic viral and bacterial infections, including HIV, Tuberculosis, leprosy and so forth.

Infestations contemplated to be treated with the nucleosides of the present invention include intracellular protozoan infestations, as well as helminth and other parasitic infestations. Again, it is especially contemplated that combinations claimed herein will be useful in treating chronic infestations.

Neoplasms contemplated to be treated include those caused by a virus, and the effect may involve inhibiting the transformation of virus-infected cells to a neoplastic state, inhibiting the spread of viruses from transformed cells to other normal cells and/or arresting the growth of virus-transformed cells.

Allergies contemplated to be treated include all IgE and IgG allergies, hyper IgE syndrome, and dermatic conditions such as atopic dermatitis. It is also contemplated that the claimed methods can be used to treat transplant rejection, (graft vs. host disease) and implant reactions.

Autoimmune diseases can be classified as either non-organ-specific or organ-specific. Non-organ-specific autoimmune diseases include rheumatoid arthritis, gout and gouty arthritis, Systemic Lupus Erythematosus (SLE), Sjogren syndrome, scleroderma, polymyositis and dermomyositis, ankylosing spondylitis, and rheumatic fever. Organ-specific autoimmune diseases are known for virtually every organ, including insulin-dependent diabetes, thyroid diseases (Graves disease and Hashimoto thyroiditis), Addison disease, and some kidney and lung diseases including allergy and asthma, multiple sclerosis, myasthenia gravis, uveitis, psoriasis, forms of hepatitis and cirrhosis, celiac disease, inflammatory bowel disease, and some types of male and female infertility. Autoimmune processes may also be stimulated by viral infections including the HIV virus, may result from rejection of transplantation, and may accompany certain tumors, or be precipitated by exposure to some chemicals.

Synthesis

Synthesis of ribavirin is well known, and synthesis of ribavirin analogs is contemplated to follow from the teachings of PCT application PCT/US97/18387 and PCT/US97/00600, the disclosures for both of which are incorporated herein by reference.

Administration

It is contemplated that nucleosides according to the present invention will be administered in any appropriate pharmaceutical formulation, and under any appropriate protocol. Preferred dosages and protocols are contemplated to be best established through experimentation with particular patients. Such experimentation need not be extensive, and it is contemplated that nucleosides will be administered in humans at between about 100 mg/day and about 5,000 mg/day. In humans and other systems, the ribavirin or other nucleoside is especially contemplated to be provided under parameters that produce a concentration of the nucleoside in a fluid containing cells expressing a B7 between about .2 :M and about 5 :M, respectively,.

Of course, where treatment of a disease is concerned, one of ordinary skill in the art will recognize that a therapeutically effective amount will vary with the infection or condition to be treated, its severity, the treatment regimen to be employed, the pharmacokinetics of the agent used, as well as the patient (animal or human) treated. Thus,

effective dosages may range from 1 mg/kg of body weight, or less, to 25 mg/kg of body weight or more. In general a therapeutically effective amount of the "second" drug is contemplated to range from slightly less than about 1 mg./kg. to about 25 mg./kg. of the patient, depending upon the nucleoside used, the condition or infection treated and the route of administration. This dosage range generally produces effective blood level concentrations of active nucleoside ranging from about 0.04 to about 100 micrograms/cc of blood in the patient. It is contemplated, however, that appropriate patient-specific regimens will be developed by administering a small amount, and then increasing the amount until either the side effects become unduly adverse, or the intended effect is achieved.

Administration of nucleosides according to the present invention may take place orally, parenterally (including subcutaneous injections, intravenous, intramuscularly, by intrasternal injection or infusion techniques), by inhalation spray, or rectally, topically and so forth, and in dosage unit formulations containing conventional non-toxic pharmaceutically acceptable carriers, adjuvants and vehicles.

It is contemplated that nucleosides according to the present invention can be formulated in admixture with a pharmaceutically acceptable carrier. For example, the nucleosides of the present invention can be administered orally as pharmacologically acceptable salts. Because the nucleosides of the present invention are mostly water soluble, they can be administered intravenously in physiological saline solution (e.g., buffered to a pH of about 7.2 to 7.5). Conventional buffers such as phosphates, bicarbonates or citrates can be used for this purpose. Of course, one of ordinary skill in the art may modify the formulations within the teachings of the specification to provide numerous formulations for a particular route of administration without rendering the compositions of the present invention unstable or compromising their therapeutic activity. In particular, the modification of the present nucleosides to render them more soluble in water or other vehicle, for example, may be easily accomplished by minor modifications (salt formulation, esterification, *etc.*) which are well within the ordinary skill in the art. It is also well within the ordinary skill of the art to modify the route of administration and dosage regimen of a particular nucleosides in order to manage the pharmacokinetics of the contemplated nucleosides for maximum beneficial effect in patients.

In certain pharmaceutical dosage forms, the pro-drug form of administered nucleosides, especially including acylated (acetylated or other) derivatives, pyridine esters and various salt forms of the present nucleosides are preferred. One of ordinary skill in the art will recognize how to readily modify the present nucleosides to pro-drug forms to facilitate delivery of active nucleosides to a target site within the host organism or patient. One of ordinary skill in the art will also take advantage of favorable pharmacokinetic parameters of the pro-drug forms, where applicable, in delivering the contemplated nucleosides to a targeted site within the host organism or patient to maximize the intended effect of the nucleoside.

In addition, contemplated nucleosides may be administered separately or together, and when administered separately this may occur in any order. The amounts of the active ingredient(s) and pharmaceutically active agent(s) and the relative timings of administration will be selected in order to achieve a desired combined therapeutic effect.

Administration routes of contemplated nucleosides may range from continuous (intravenous drip) to several oral administrations per day (for example, Q.I.D.) and may include oral, topical, parenteral, intramuscular, intravenous, sub-cutaneous, transdermal (which may include a penetration enhancement agent), buccal and suppository administration, among other routes of administration.

In treatments according to the present invention, a therapeutically effective amount of a nucleoside is preferably intimately admixed with a pharmaceutically acceptable carrier according to conventional pharmaceutical compounding techniques to produce a dose. A carrier may take a wide variety of forms depending on the form of preparation desired for administration, e.g., oral or parenteral. In preparing pharmaceutical compositions in oral dosage form, any of the usual pharmaceutical media may be used. Thus, for liquid oral preparations such as suspensions, elixirs and solutions, suitable carriers and additives including water, glycols, oils, alcohols, flavoring agents, preservatives, coloring agents and the like may be used. For solid oral preparations such as powders, tablets, capsules, and for solid preparations such as suppositories, suitable carriers and additives including starches, sugar carrier, such as dextrose, mannitol, lactose and related carriers, diluents, granulating agents, lubricants, binders, disintegrating agents and the like may be used. If desired, the tablets or capsules may be enteric-coated or sustained release by standard techniques.

For parenteral formulations, the carrier will usually comprise sterile water or aqueous sodium chloride solution, though other ingredients including those that aid dispersion may be included. Of course, where sterile water is to be used and maintained as sterile, the compositions and carriers must also be sterilized. Injectable suspensions may also be prepared, in which case appropriate liquid carriers, suspending agents and the like may be employed.

It will also be appreciated that in general, the most preferred uses according to the present invention are those in which the active nucleosides are relatively less cytotoxic to the non-target host cells and relatively more active against the target. In this respect, it may also be advantageous that L-nucleosides may have increased stability over D-nucleosides, which could lead to better pharmacokinetics. This result may attain because L-nucleosides may not be recognized by enzymes, and therefore may have longer half-lives.

Thus, methods have been disclosed that employ ribavirin or other nucleosides to advantageously modulate a B7 molecular marker.. While specific embodiments have been disclosed herein, the scope of the invention is not be limited except through interpretation of the appended claims.

CLAIMS

What is claimed is:

1. A method of modulating a response of an immune system to a challenge comprising:

correlating the challenge with an effect on a B7 marker;

correlating application of a nucleoside within a concentration range with modulation of the B7 molecular marker that is inverse to the effect; and

presenting the immune system with the nucleoside within the concentration range.

2. The method of claim 1 wherein the challenge comprises an allergen.

3. The method of claim 1 wherein the challenge comprises a neoplasm.

4. The method of claim 1 wherein the challenge comprises a virus.

5. The method of claim 1 wherein the challenge comprises a bacterium.

6. The method of claim 1 wherein the challenge comprises an infestation.

7. The method of claim 1 wherein the challenge comprises an autoimmune reaction.

8. The method of any of claims 1 – 8 wherein the molecular marker is B7-1.

9. The method of any of claims 1 – 8 wherein the molecular marker is B7-2.

10. The method of any of claims 1 – 8 wherein the nucleoside is Ribavirin.

11. The method of any of claims 1 – 8 wherein the nucleoside is a Ribavirin analog.

12. The method of claim 1 wherein the challenge is selected from the group consisting of an allergen, a microbe, a neoplasm, an infestation, and autoimmune reaction, the molecular marker is B7-1, and the nucleoside is Ribavirin.

13. The method of claim 1 wherein the challenge is selected from the group consisting of an allergen, a microbe, a neoplasm, an infestation, and autoimmune reaction, the molecular marker is B7-2, and the nucleoside is Ribavirin.

14. The method of claim 1 wherein the challenge is selected from the group consisting of an allergen, a microbe, a neoplasm, an infestation, and autoimmune reaction, the molecular marker is B7-1, and the nucleoside is not Ribavirin.

15. The method of any of claims 12 – 14 wherein the concentration range is between about .2 :M and about 5 :M, respectively, in a fluid containing cells expressing the B7 marker.

16. The method of any of claims 1 – 8 further comprising correlating the challenge an increase in Type 2 response, and correlating application of the nucleoside with a decrease in Type 2 response.

AMENDED CLAIMS

[received by the International Bureau on 13 June 2000 (13.06.00); original claims 8-11 and 15-16 amended; remaining claims unchanged (2 pages)]

1. A method of modulating a response of an immune system to a challenge comprising:

correlating the challenge with an effect on a B7 marker:

correlating application of a nucleoside within a concentration range with modulation of the B7 molecular marker that is inverse to the effect; and

presenting the immune system with the nucleoside within the concentration range.

2. The method of claim 1 wherein the challenge comprises an allergen.

3. The method of claim 1 wherein the challenge comprises a neoplasm.

4. The method of claim 1 wherein the challenge comprises a virus.

5. The method of claim 1 wherein the challenge comprises a bacterium.

6. The method of claim 1 wherein the challenge comprises an infestation.

7. The method of claim 1 wherein the challenge comprises an autoimmune reaction.

8. The method of any one of claims 1 – 8 wherein the molecular marker is B7-1.

9. The method of any one of claims 1 – 8 wherein the molecular marker is B7-2.

10. The method of any one of claims 1 – 8 wherein the nucleoside is Ribavirin.

11. The method of any one of claims 1 – 8 wherein the nucleoside is a Ribavirin analog.

12. The method of claim 1 wherein the challenge is selected from the group consisting of an allergen, a microbe, a neoplasm, an infestation, and autoimmune reaction, the molecular marker is B7-1, and the nucleoside is Ribavirin.

13. The method of claim 1 wherein the challenge is selected from the group consisting of an allergen, a microbe, a neoplasm, an infestation, and autoimmune reaction, the molecular marker is B7-2, and the nucleoside is Ribavirin.

14. The method of claim 1 wherein the challenge is selected from the group consisting of an allergen, a microbe, a neoplasm, an infestation, and autoimmune reaction, the molecular marker is B7-1, and the nucleoside is not Ribavirin.

15. The method of any one of claims 12 – 14 wherein the concentration range is between about .2 μM and about 5 μM, respectively, in a fluid containing cells expressing the B7 marker.

16. The method of any one of claims 1 – 8 further comprising correlating the challenge an increase in Type 2 response, and correlating application of the nucleoside with a decrease in Type 2 response.

Figure 1
Correlation Of Specific Diseases
With B7-1 and B7-2 Expression

Cancer type	B7-1	B7-2
Hepatcytic carcinoma	Down	Down
Gastric carcinoma (primary)	Up	Up
Gastric carcinoma (metastatic)	Down	Up
Head & Neck (primary and established)	Down	Down
Acute Myeloid Leukemia	Down	Up
Autoimmune disease		
Multiple sclerosis	Up	Up
Asthma	Neutral	Up
Inflammatory bowel syndrome	Up	Up

Figure 2
Correlation Of Various Disease Types With
Type 1, Type 2, B7-1, and B7-2 Expression

Type/Response	Type 1	Type 2	B7-1	B7-2
Autoimmune disease	Up (organ specific AIDS)	Up (systemic AIDS)	Up	Up/down
Cancer	Down	Up	Up/down	Up/down
Bacterial infection	Up	Up	Up	Up
Viral infection	Up	Up	Up	Up

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/30490

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :A61K 31/70; C07H 19/052.
US CL :514/43; 536/28.7.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/43; 536/28.7.

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

File CA structure search.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,767,097 A (TAM) 16 June 1998, see entire document.	1-4, 6-7, 12-13 and 15
Y	WO 98/16186 A2 (ICN PHARMACEUTICALS.) 23 April 1998, see entire document.	1-7, 12-15

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 13 MARCH 2000	Date of mailing of the international search report 17 APR 2000
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer LAWRENCE ERIC CRANE Telephone No. (703) 308-1235 JOYCE BRIDGERS PARALEGAL SPECIALIST CHEMICAL MATRIX

Form PCT/ISA/210 (second sheet) (July 1998)☆

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/30490

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 8-11 and 16
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.